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(FILE 'HOME' ENTERED AT 18:11:26 ON 30 NOV 2005)

FILE 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DGENE, DISSABS, DRUGB, DRUGMONOG2, ...' ENTERED AT 18:12:08 ON 30 NOV 2005

L1 734 S (STAUDINGER OR PHOSPHINE) AND (FARNESYL? OR PRENYL?)
L2 349 S L1 AND AZID?
L3 287 S L2 AND CELL
L4 256 DUP REM L3 (31 DUPLICATES REMOVED)
L5 251 S L4 AND (PROTEIN OR ENZYME)
L6 249 S L5 AND (PURIF? OR ISOLAT? OR SEPARAT? OR CHROMATOGRAPH?)
L7 193 S L6 AND ((PURIF? OR ISOLAT? OR SEPARAT? OR CHROMATOGRAPH?) (S)
L8 5 S L7 AND ((FARNESYL? OR PRENYL?) (S) (AZID?))

=> d l8 1-5 ibib abs

L8 ANSWER 1 OF 5 DGENE COPYRIGHT 2005 The Thomson Corp on STN

ACCESSION NUMBER: ADZ88868 peptide DGENE

TITLE: Detecting an isoprenylated **protein** in a **cell**, comprises contacting the **cell** with an isoprenyl **azide** substrate, in which an **azide** from a substrate is incorporated into the **protein**, and detecting using a **Staudinger** reaction.

INVENTOR: Zhao Y; Falck J R

PATENT ASSIGNEE: (ZHAO-I)ZHAO Y.

(FALC-I) FALCK J R.

PATENT INFO: US 2005106627 A1 20050519 18

APPLICATION INFO: US 2003-715329 20031117

PRIORITY INFO: US 2003-715329 20031117

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2005-394577 [40]

DESCRIPTION: RC peptide SEQ ID NO:1.

AN ADZ88868 peptide DGENE

AB The invention relates to a method (M1) for detecting a first isoprenylated **protein** in a **cell**. (M1) comprises: (a) obtaining a synthetic isoprenyl **azide** substrate of a **protein** in the **cell**; (b) contacting the **cell** under conditions, where the **cell** takes up and incorporates into the **protein** an **azide** from the substrate; and (c) detecting the first **protein** from proteins produced by the **cell** with a **phosphine** capture reagent by the **Staudinger** reaction. Also described: (1) labeling (M2) a **protein** in a **cell** comprising preparing a synthetic substrate of the **protein** comprising at least a first **azide**, and contacting the **cell** under conditions where the synthetic substrate is taken up and incorporated into the **protein** and the **protein** is labeled with the first **azide**; and (2) a compound (C1) having the molecular formula (I),

(II) or (III). (M1) is useful for detecting at least a first isoprenylated **protein** such as Ras, in a **cell**. (M2) and C1 are useful for labeling a **protein** in a **cell**. (M1) enables detection and **isolation** of isoprenylated proteins with high yield, high specificity and low contamination without harsh treatment of proteins. (M1) allows uniformly chemoselective enrichment of proteins containing an **azide** group using **phosphine** capture reagents. (M1) allows efficient analysis of dynamic **farnesylation** modification. (M1) enables identification and quantification of low-abundant **farnesylated** proteins. (M1) is suitable for the characterization of biological functions and identification of **farnesylated protein** targets in response to drugs. The present sequence represents an RC peptide, which is a CAAX-box containing peptide used in an example from the present invention to determine whether **farnesyltransferase** can be used FPP **azide** as a substrate for **protein** **farnesylation**.

L8 ANSWER 2 OF 5 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2004363657 EMBASE
 TITLE: A tagging-via-substrate technology for detection and proteomics of **farnesylated** proteins.
 AUTHOR: Kho Y.; Kim S.C.; Jiang C.; Barma D.; Kwon S.W.; Cheng J.; Jaunbergs J.; Weinbaum C.; Tamanoi F.; Falck J.; Zhao Y.
 CORPORATE SOURCE: Y. Zhao, Department of Biochemistry, Univ. Texas Southwestern Med. Ctr., Dallas, TX 75390-9038, United States. yzhao@biochem.swmed.edu
 SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (24 Aug 2004) Vol. 101, No. 34, pp. 12479-12484.
 Refs: 34
 ISSN: 0027-8424 CODEN: PNASA6
 COUNTRY: United States
 DOCUMENT TYPE: Journal; Article
 FILE SEGMENT: 029 Clinical Biochemistry
 LANGUAGE: English
 SUMMARY LANGUAGE: English
 ENTRY DATE: Entered STN: 20040924
 Last Updated on STN: 20040924

AB A recently developed proteomics strategy, designated tagging-via-substrate (TAS) approach, is described for the detection and proteomic analysis of **farnesylated** proteins. TAS technology involves metabolic incorporation of a synthetic **azido-farnesyl** analog and chemoselective derivatization of **azido-farnesyl** -modified proteins by an elegant version of **Staudinger** reaction, pioneered by the Bertozzi group, using a biotinylated **phosphine** capture reagent. The resulting **protein** conjugates can be specifically detected and/or affinity-purified by streptavidin-linked horseradish peroxidase or agarose beads, respectively. Thus, the technology enables global profiling of **farnesylated** proteins by enriching **farnesylated** proteins and reducing the complexity of **farnesylation** subproteome. **Azido-farnesylated** proteins maintain the properties of **protein** **farnesylation**, including promoting membrane association, Ras-dependent mitogen-activated **protein** kinase kinase activation, and inhibition of lovastatin-induced apoptosis. A proteomic analysis of **farnesylated** proteins by TAS technology revealed 18 **farnesylated** proteins, including those with potentially novel **farnesylation** motifs, suggesting that future use of this method is likely to yield novel insight into **protein** **farnesylation**. TAS technology can be extended to other posttranslational modifications, such as geranylgeranylation and myristoylation, thus providing powerful tools for detection, quantification, and proteomic

analysis of posttranslationally modified proteins.

L8 ANSWER 3 OF 5 FEDRIP COPYRIGHT 2005 NTIS on STN

ACCESSION NUMBER: 2005:185640 FEDRIP

NUMBER OF REPORT: CRISP 1R21CA107943-01

RESEARCH TITLE: A Novel Proteomics Technology for **Protein Farnesylation**

STAFF: Principal Investigator: ZHAO, YINGMING; YZHAO@BIOCHEM.SWMED.EDU, UNIVERSITY OF TEXAS SW MED CTR, 5323 HARRY HINES BLVD, DALLAS, TX 75390

PERFORMING ORGN: UNIVERSITY OF TEXAS SW MED CTR/DALLAS, DALLAS, TEXAS

SUPPORTING ORGN: Supported By: NATIONAL CANCER INSTITUTE

PROJECT START DATE: 2006 (/07/04)

FISCAL YEAR: 2004

ESTD COMPLETION DATE: 2005 (/31/05)

FUNDING: New Award (Type 1)

FILE SEGMENT: National Institutes of Health

SUM DESCRIPTION (provided by applicant): The identification and characterization of changes in the level of **farnesylated** proteins in response to the treatment of **farnesyltransferase** inhibitors (FTIs), a newly introduced family of antitumor agents currently undergoing clinical evaluation, represents a major scientific challenge. Extant proteomics methods are limited to quantifying a few thousands of the most abundant proteins and, therefore, are unsuitable for the targeted profiling of less abundant **farnesylated** expression. The major goal of this application is to develop and validate a powerful technology, Tagging via **Azido** Substrate (TAS), for the efficient **isolation** of **farnesylated** proteins. This technology will then be applied to the proteomics analysis of **farnesylated** proteins in physiologically relevant models. The TAS technology involves the introduction of a synthetic **azide**-modified **farnesyl** substrate, either **farnesyl azide** diphosphate (FPP-**azide**) or **farnesyl azide** alcohol (F-**azide**-OH), which replaces the natural substrate during cellular **protein farnesylation**. The resulting **farnesyl-azide** (F-**azide**)-modified proteins will be affinity-**purified** through an **azide**-specific conjugation reaction (Staudinger reaction) using a **phosphine** capture reagent linked to photo-cleavable beads, which can then be released by UV light-induced photo-cleavage. Since affinity **purification** relies on covalent bonding resulting from a specific conjugation reaction between an **azide** and **phosphine** capture reagent, other proteins without the F-**azide** modification can be effectively removed by thorough washing. Thus, the TAS technology will allow **farnesylated** proteins to be **isolated** with high yield, high specificity, and low contamination. The initial focus of the R21 portion of this proposal is on the development of TAS technology and its application to the **isolation** of **farnesylated** proteins. These studies will be extended subsequently to geranylgeranylated proteins. The R33 proposal will aim at applications of the TAS technology to the identification of novel FTI targets. These studies will provide fundamental information for the understanding of molecular mechanisms of FTI functions and are likely to identify novel targets for antitumor drug design.

L8 ANSWER 4 OF 5 IFIPAT COPYRIGHT 2005 IFI on STN

AN 10867911 IFIPAT;IFIUDB;IFICDB

TITLE: METHODS AND COMPOSITIONS FOR TAGGING VIA **AZIDO** SUBSTRATES

INVENTOR(S): Falck; John R., University Park, TX, US
Zhao; Yingming, Dallas, TX, US

PATENT ASSIGNEE(S): Unassigned

AGENT: FULBRIGHT & JAWORSKI L.L.P., 600 CONGRESS AVE., SUITE 2400, AUSTIN, TX, 78701, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2005106627	A1	20050519
APPLICATION INFORMATION:	US 2003-715329		20031117
FAMILY INFORMATION:	US 2005106627		20050519
DOCUMENT TYPE:	Utility		
	Patent Application - First Publication		
FILE SEGMENT:	CHEMICAL		
	APPLICATION		

GOVERNMENT INTEREST:

The government may own rights in the invention pursuant to grant number CA 85146 and GM 31278 from the NIH.

NUMBER OF CLAIMS: 32 4 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1. Schematic representation of one embodiment of the TAS technology for solid-phase-isolation of **azide-labeled farnesylated** proteins. (A) **Farnesyltransferase**-catalyzed enzymatic reaction using FPP or FPP **azide** as a substrate. (B) Chemical structures of natural FPP, and a FPP-**azide** and the corresponding alcohols, F-OH (farnesol) and F-**azide**-OH. (C) A **Staudinger** conjugation reaction between a **phosphine** and an **azide**-containing molecule. (D) The structure of a biotinylated **phosphine** capture reagent and its reaction product with an **azido-farnesylated protein**. (E) Experimental procedure for the **isolation** of F-**azide** modified proteins. **Protein i** and **ii**, unmodified proteins; *****protein*** iii**, a **protein** modified by natural **farnesyl** group; and **protein iv**, F-**azide** modified proteins. Only F-*****azide***** modified **protein iv** is **purified** and released by UV light-induced photocleavage.

FIG. 2. FPP **azide** is a substrate for in vitro **farnesylation** reaction. MALDI-TOF mass spectra of RC peptide before (A) and after (B) in vitro **farnesylation** reaction. One hundred pmol RCpeptide (KKFFCAIS) were mixed with 3000 pmol FPP **azide** and 35 pmol FTase in 7 μ l reaction buffer and incubated at 30 degrees C. for 10 h. The F-**azide** modified RC-peptide was confirmed by mass spectrometry (m/z, 1191).

FIG. 3. Detection of F-**azide** modified Ras and HDJ-2 by mobilityshift assay. Mobility-shift assay of Ras (A) and HDJ-2 (B). COS1 cells were labeled with the indicated compounds for 24 h, the **cell** lysate were resolved in SDS-PAGE and probed using anti-Ras and anti-HDJ-2 antibodies, respectively. The letters, "u" and "p", indicate the unmodified and isoprenylated forms of the **protein**, respectively.

FIG. 4. Global detection of F-**azide** modified proteins by Western blotting analysis. COS cells were labeled with DMSO, or FPP N3 with or without lovastatin, as indicated, for 24 h. The **protein** lysates from the cells were precipitated by acetone/TCA method, redissolved in a buffer containing 2% SDS and PBS. The resulting solution was conjugated to biotinylated **phosphine** capture reagents or not, precipitated by acetone/TCA method again. The **protein** pellet was redissolved in 1xSDS sample buffer, resolved in SDS-PAGE, and detected by Western blotting analysis using streptavidin-conjugated HRP with or without biotin.

AB The invention provides methods and compositions for **azide** tagging of biomolecules. In one embodiment of the invention, proteins are tagged by metabolic incorporation of **prenylated azido** -analog substrates. Examples of such analogs are **azido** farnesyl diphosphate and **azido** farnesyl alcohol. The **azido** moiety in the resulting modified proteins provides an affinity tag, which can be chemoselectively captured by an **azide**-specific conjugation reaction, such as the **Staudinger** reaction, using a **phosphine** capture reagent. When the capture agent is biotinylated, the resulting conjugates can be detected and affinity-**purified** by streptavidin-linked-HRP and streptavidinconjugated agarose beads, respectively. The invention allows detection and

isolation of proteins with high yield, high specificity, and low contamination without harsh treatment of proteins.

CLMN 32 4 Figure(s).

FIG. 1. Schematic representation of one embodiment of the TAS technology for solid-phase-isolation of azide-labeled farnesylated proteins. (A) Farnesyltransferase-catalyzed enzymatic reaction using FPP or FPP azide as a substrate. (B) Chemical structures of natural FPP, and a FPP-azide and the corresponding alcohols, F-OH (farnesol) and F-azide-OH. (C) A Staudinger conjugation reaction between a phosphine and an azide-containing molecule. (D) The structure of a biotinylated phosphine capture reagent and its reaction product with an azido-farnesylated protein. (E) Experimental procedure for the isolation of F-azide modified proteins. Protein i and ii, unmodified proteins; protein iii, a protein modified by natural farnesyl group; and protein iv, F-azide modified proteins. Only F-azide modified protein iv is purified and released by UV light-induced photocleavage.

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L8 ANSWER 5 OF 5 USPATFULL on STN

ACCESSION NUMBER: 2004:267776 USPATFULL

TITLE: Methods and compositions for peptide and protein labeling

INVENTOR(S): Ting, Alice Y., Allston, MA, UNITED STATES

PATENT ASSIGNEE(S): Massachusetts Institute of Technology, Cambridge, MA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2004209317	A1	20041021
APPLICATION INFO.:	US 2004-754911	A1	20040109 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-438939P	20030109 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	Maria A. Trevisan, Wolf, Greenfield & Sacks, P.C., 600	

Atlantic Avenue, Boston, MA, 02210

NUMBER OF CLAIMS: 69
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 2729

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions and methods of use thereof for labeling peptide and proteins in vitro or in vivo. The methods described herein employ biotin ligase mutants and biotin analogs recognized by such mutants.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> file chemistry

=> s 18

L9 1 L8

=> d 19 ibib abs

L9 ANSWER 1 OF 1 FEDRIP COPYRIGHT 2005 NTIS on STN

ACCESSION NUMBER: 2005:185640 FEDRIP

NUMBER OF REPORT: CRISP 1R21CA107943-01

RESEARCH TITLE: A Novel Proteomics Technology for **Protein Farnesylation**

STAFF: Principal Investigator: ZHAO, YINGMING; YZHAO@BIOCHEM.SWMED.EDU, UNIVERSITY OF TEXAS SW MED CTR, 5323 HARRY HINES BLVD, DALLAS, TX 75390

PERFORMING ORGN: UNIVERSITY OF TEXAS SW MED CTR/DALLAS, DALLAS, TEXAS

SUPPORTING ORGN: Supported By: NATIONAL CANCER INSTITUTE

PROJECT START DATE: 2006 (/07/04)

FISCAL YEAR: 2004

ESTD COMPLETION DATE: 2005 (/31/05)

FUNDING: New Award (Type 1)

FILE SEGMENT: National Institutes of Health

SUM DESCRIPTION (provided by applicant): The identification and characterization of changes in the level of **farnesylated** proteins in response to the treatment of **farnesyltransferase** inhibitors (FTIs), a newly introduced family of antitumor agents currently undergoing clinical evaluation, represents a major scientific challenge. Extant proteomics methods are limited to quantifying a few thousands of the most abundant proteins and, therefore, are unsuitable for the targeted profiling of less abundant **farnesylated** expression. The major goal of this application is to develop and validate a powerful technology, Tagging via **Azido** Substrate (TAS), for the efficient **isolation** of **farnesylated** proteins. This technology will then be applied to the proteomics analysis of **farnesylated** proteins in physiologically relevant models. The TAS technology involves the introduction of a synthetic **azide**-modified **farnesyl** substrate, either **farnesyl azide** diphosphate (FPP-**azide**) or **farnesyl azide** alcohol (F-**azide**-OH), which replaces the natural substrate during cellular **protein farnesylation**. The resulting **farnesyl-azide** (F-**azide**)-modified proteins will be affinity-purified through an **azide**-specific conjugation reaction (Staudinger reaction) using a **phosphine** capture reagent linked to photo-cleavable beads, which can then be released by UV light-induced photo-cleavage. Since affinity **purification** relies on covalent bonding resulting from a specific conjugation reaction between an **azide** and **phosphine** capture reagent, other proteins

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF

LOGOFF? (Y)/N/HOLD:y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

61.73

318.71

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